

UNCLASSIFIED

AD NUMBER
AD073411
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; Sep 1955. Other requests shall be referred to the U.S. Army Medical Research Laboratory, Fort Knox, KY.
AUTHORITY
USAMRL ltr, 26 Feb 1970

THIS PAGE IS UNCLASSIFIED

20030527026

AMRL-209

AD 073411 001



REPORT NO. 209

**SPECIAL CUVETTES FOR SPECTROPHOTOMETRIC
MEASUREMENTS OF MICRO-ORGANISMS AND LIVING TISSUES***

by

Charles W. McKeehan and Irven C. Graham

from

Biophysics Department
ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY

1 September 1955

*Subtask under Environmental Physiology, AMRL Project No. 6-
64-12-028, Subtask, Biophysical Studies of Skin.

AMRL-209

AD 073411 001



Report No. 209
Project No. 6-64-12-028
Subtask AMRL S-14
MEDEA

ABSTRACT

SPECIAL CUVETTES FOR SPECTROPHOTOMETRIC MEASUREMENTS OF MICRO-ORGANISMS AND LIVING TISSUES

OBJECT

To construct cuvettes for spectrophotometry in which the biological conditions can be modified during the measurement.

RESULTS AND CONCLUSIONS

The cuvettes gave perfect service in combination with a modified Beckman DR spectrophotometer and with a General Electric recording spectrophotometer.

RECOMMENDATIONS

None.

Submitted 19 August 1955 by:
Charles W. McKeehan, SP2
Irven C. Graham

APPROVED: _____

Ray G. Daggs
RAY G. DAGGS
Director of Research

APPROVED: _____

William W. Cox
WILLIAM W. COX
Lt Colonel, MC
Commanding

SPECIAL CUVETTES FOR SPECTROPHOTOMETRIC MEASUREMENTS OF MICRO-ORGANISMS AND LIVING TISSUES

I. INTRODUCTION

In the course of the study of the spectral reflectance of human and animal skin over a wide spectral range (1-5) the integrating sphere (6) and its combination with various spectrophotometers, originally not equipped with it, (1, 7, 8) has been thoroughly investigated. Jacquez has demonstrated that this improved method of measuring spectral reflectance is versatile and applicable also to spectrophotometric absorption measurements on turbid and even opaque systems. He has obtained absorption spectra of living tissues and micro-organisms which compare favorably with those obtained with more elaborate and sensitive techniques (9). It is expected that the improved method will be useful in other fields, such as the study of the kinetics of respiratory enzymes, photosynthesis, and photoreactivation.

In Jacquez's experiments two special cuvettes, one for suspensions of micro-organisms, and a slightly different one for tissue samples, backed by a block of MgCO_3 during the reflectance measurement were used (9). They permitted changing the biological conditions of the systems during measurement without removing the sample from the cuvette. To facilitate further applications of the improved method, the cuvettes are described here in detail.

II. DESIGN, FUNCTION AND ASSEMBLY

The basic design for the two cuvettes is as follows. Two plates of fused quartz, each of which was 1/16 in. thick and 10 cm in diameter, were held together by 4 small screw clamps placed along the periphery. In one of the cuvettes used, a Plexiglass spacer ring with a 5.4 cm inner diameter was sealed tightly between the two quartz plates by applying a thin layer of stopcock grease or petrolatum on each face of the ring; this cuvette was used to measure the reflectance of suspensions. In the other cuvette, a Plexiglass spacer ring with a 7 cm inner diameter was sealed between the plates; this cuvette was used to measure the reflectance of tissue samples. Spacers of different thicknesses (one-fourth to one-eighth in.) can be used to vary the length of the light path or to accommodate tissue samples of different thickness.

The difference between the two types of cuvettes is in the design of the spacers. The spacer used for the suspension cell, shown in

Figure 1A, has four radial bores holding hypodermic needles (15 gauge, both ends cut off). The needles are sealed in with chloroform. They provide openings for admitting the sample into the cell, for adding chemicals and for the inlet and outlet of gases. The bubbling space at the gas outlet prevents the liquid from being forced out. The suspension cuvette is assembled first and then filled with the system to be measured.

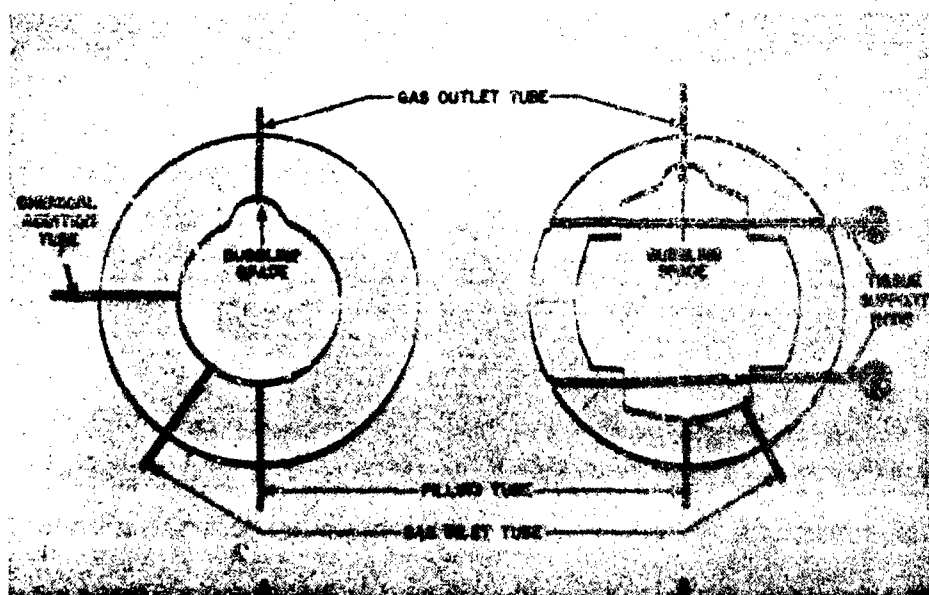


FIG. 1 - THE SPACERS FOR THE SUSPENSION (A) AND TISSUE (B) CUVETTES.

The spacer for the tissue cuvette, with a slightly different shape as shown in Figure 1B, has only three radial bores with sealed-in hypodermic needles. In addition, two parallel nickel-silver rods, 1/16 in. thick, are sealed into the spacer.

Two opposite edges of the tissue sample (usually rectangular) can be fastened to the rods with at least four 11 mm Michelle clips on each edge holding the sample flat. The rods and clips serve as electrodes for electrical stimulation of the sample (9). For most tissue samples a one-fourth in. spacer is convenient. This cuvette must be assembled after the tissue sample is mounted. It is shown, containing a strip of abdominal rat muscle, in Figure 2.

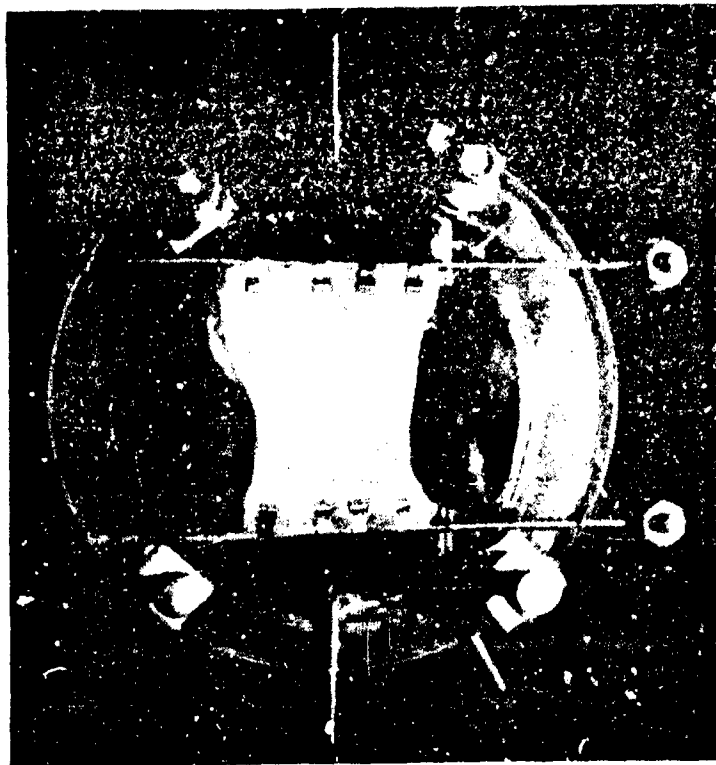


FIG. 2 - THE ASSEMBLED TISSUE CUVETTE

III. DISCUSSION AND CONCLUSIONS

The two cuvettes gave perfect service in combination with the modified (8) Beckman DR spectrophotometer. They were also used in combination with the GE recording spectrophotometer, in this case without the screw clamps because the spring-activated covers for the openings of the integrating sphere supplied enough pressure for holding the cell parts tight.

IV. RECOMMENDATIONS

None.

V. BIBLIOGRAPHY

1. Jacquez, J. A., J. T. Randall, M. Goodman, W. McKeehan, J. M. Dimitroff and H. F. Kuppenheim. The Spectral Reflectance of Human Skin in the Region 235-1000 m μ . AMRL Report No. 159, Fort Knox, Kentucky, 1 December 1954.
2. Jacquez, J. A., J. Huss, W. McKeehan, J. M. Dimitroff and H. F. Kuppenheim. The Spectral Reflectance of Human Skin in the Region 0.7-2.6 μ . AMRL Report No. 189, Fort Knox, Kentucky, 18 April 1955.
3. Jacquez, J. A., H. F. Kuppenheim, J. M. Dimitroff, W. McKeehan and J. Huss. The Spectral Reflectance of Human Skin in the Region 235-700 m μ . AMRL Report No. 193, Fort Knox, Kentucky, 20 April 1955.
4. Dimitroff, J. M., J. A. Jacquez, and H. F. Kuppenheim. Spectral Reflectance of the Skin of Rats and Rabbits in the Region 420-1000 m μ . AMRL Report No. 177, Fort Knox, Kentucky, 2 March 1955.
5. Dimitroff, J. M., H. F. Kuppenheim, P. M. Melotti, J. Huss, I. C. Graham, W. McKeehan and D. W. Swanson. Spectral Reflectance of the Skin of Rats, Rabbits and Hairless Mice in the Regions 243-700 m μ and .707-2.660 μ . AMRL Report No. 203, Fort Knox, Kentucky, 19 July 1955.
6. Jacquez, J. A., and H. F. Kuppenheim. The Theory of the Integrating Sphere. AMRL Report No. 150, Fort Knox, Kentucky, 26 November 1954.

7. Jacques, J. A., W. McKeehan, J. Huss, J. M. Dimitroff and H. F. Kuppenheim. An Integrating Sphere for Measuring Diffuse Reflectance in the Near Infrared. AMRL Report No. 188, Fort Knox, Kentucky, 14 April 1955.
8. Jacques, J. A., W. McKeehan, J. Huss, J. M. Dimitroff and H. F. Kuppenheim. An Integrating Sphere for the Measurement of Reflectance with the Beckman Model DR Recording Spectrophotometer. AMRL Report No. 192, Fort Knox, Kentucky, 19 April 1955.
9. Jacques, J. A., with the technical assistance of C. W. McKeehan, J. D. Huss, I. C. Graham, J. M. Melotti, Dimitroff and D. W. Swanson. The Use of the Integrating Sphere in Spectrophotometric Studies of Living Tissues and Micro-Organisms. AMRL Report No. 194, Fort Knox, Kentucky, 15 May 1955.

DISTRIBUTION LIST FOR AMRL REPORTS

Category (A)

<u>ARMY</u>	<u>Copies</u>	<u>ARMY</u>	<u>Copies</u>
The Surgeon General Department of the Army Main Navy Building Washington 25, D. C. ATTN: Chief, Research and Development Division	10	Supreme Headquarters of the Allied Powers in Europe Medical Section Requencourt, France	2
Director Armed Forces Institute of Pathology Walter Reed Army Medical Center Washington 12, D. C.	1	Office, Chief of Army Field Forces Fort Monroe, Virginia	1
Army Library Room 1A 522, The Pentagon Washington 25, D. C. ATTN: National Defense Review	1	Walter H. Moursund, Jr., Colonel GS Office of the Army Attache, Box 36 U. S. Navy 100 Fleet Post Office New York, New York	2
Headquarters Army Medical Service Graduate School Walter Reed Army Medical Center Washington 12, D. C.	1	Commanding General Headquarters, QM R&D Command QM R&D Center, U. S. Army Natick, Massachusetts	3
Chief, Human Relations & Research Branch Military Personnel Management Division Office of Assistant Chief of Staff Department of the Army Room 2C724, The Pentagon Washington 25, D. C.	2	Commanding Officer Medical Nutrition Laboratory 9937 TU U. S. Army Fitzsimmons Army Hospital Denver 7, Colorado	1
Office Assistant Chief of Staff, G4 Department of the Army The Pentagon Washington 25, D. C. ATTN: Research & Development	1	Headquarters 406th Medical General Laboratory APO 500, c/o Postmaster San Francisco, California	1
Commanding Officer Chemical Corps Medical Laboratories Army Chemical Center, Maryland ATTN: Chief, Technical Information Branch	1	Headquarters Camp Detrick Frederick, Maryland ATTN: Technical Library	1
Army Environmental Health Laboratory Building No. 1235 Army Chemical Center, Maryland	1	Scientific Publications and Reports Office QM Food and Container Institute for the Armed Forces 1819 West Pershing Road Chicago 9, Illinois	1
Commandant Marine Corps Schools Quantico, Virginia ATTN: Library & Record Section, MCEG	1	Commanding Officer Arctic Test Branch, OCAFF (AAU 8576) APO 733, c/o Postmaster Seattle, Washington	1
Medical Field Service School Fort Sam Houston, Texas	2	Research and Development Division Office, Quartermaster General Room 2102, Building 'A' Washington 25, D. C.	1
Library, Army War College Carlisle Barracks Pennsylvania	1	Armed Forces Staff College Norfolk, Virginia	1
		Library, Quartermaster School Fort Lee, Virginia	1
<u>NAVY</u>		<u>NAVY</u>	
U. S. Naval School of Aviation Medicine 1 U. S. Naval Air Station Pensacola, Florida		Commanding Officer U. S. Naval Air Development Center Johnsville, Pennsylvania ATTN: Aviation Medical Acceleration Lab	1

Category (A) Continued

<u>NAVY</u>	<u>Copies</u>	<u>NAVY</u>	<u>Copies</u>
Commanding Officer Naval Medical Research Institute National Naval Medical Center Bethesda 14, Maryland	1	Officer in Charge U. S. Naval Medical Research Lab U. S. Naval Submarine Base New London, Connecticut ATTN: Librarian	1
Chief, Bureau of Medicine & Surgery Department of the Navy Washington 25, D. C.	1	Special Assistant for Bio Sciences Office of Naval Research Department of the Navy Washington 25, D. C.	1
Commanding Officer Naval Medical Field Research Laboratory Marine Barracks Camp LeJeune, N. C.	1		
<u>AIR FORCE</u>		<u>AIR FORCE</u>	
Commandant USAF School of Aviation Medicine Randolph Air Force Base Randolph Field, Texas ATTN: Research Secretariat	1	Department of the Air Force Headquarters USAF Director, Research & Development DCS/D Washington 25, D. C. ATTN: AFDRD-HF	1
Commander HQ Wright Air Development Center Wright-Patterson Air Force Base, Ohio ATTN: Aero Medical Laboratory (WCRD) Directorate of Research	1	Arctic Aeromedical Laboratory APO 731, c/o Postmaster Seattle, Washington ATTN: Librarian, AAL	1
Director Air University Library Maxwell Air Force Base, Alabama	1	Commander Air Research & Development Command ATTN: RDTRH PO 1395 Baltimore 3, Maryland	1
<u>OTHER AGENCIES</u>		<u>OTHER AGENCIES</u>	
Exchange & Gift Division Library of Congress Washington 25, D. C.	1	Armed Services Technical Information Agency Document Service Center Knott Building Dayton 2, Ohio ATTN: DSC-SD32	5
Director, Armed Forces Medical Library 7th Street & Independence Ave, SW Washington 25, D. C. ATTN: Acquisitions Division	1	National Science Foundation 1520 H Street, NW Washington, D. C.	1
Operations Research Office The Johns Hopkins University 7100 Connecticut Ave Chevy Chase, Maryland ATTN: Librarian	1	Department of Physiology University of Rochester School of Medicine and Dentistry 260 Crittenden Blvd Rochester, New York	1
National Institutes of Health Division of Research Grants Bethesda 14, Maryland	2	National Research Council Division of Medical Science 2101 Constitution Ave Washington, D. C.	1
<u>FOREIGN ADDRESSES</u>		<u>FOREIGN ADDRESSES</u>	
Defense Research Member Canadian Joint Staff (W) 2001 Connecticut AVE., NW Washington 8, D. C.	2	Officer in Charge Office of Naval Research Branch Office Navy 100, Fleet Post Office New York, New York	10
RCAMC Liaison Officer Surgeon General's Office Department of the Army Room 2842, Main Navy Building Washington 25, D. C.	2	THRU: The Foreign Service of the United States of America United States Army Liaison Office American Consulate General Singapore (for Clearance and forwarding to:) The Deputy Director of Army Health Far East Land Forces Singapore	1

Category (A) Continued

FOREIGN ADDRESSES

Copies

Tropical Research Medical Laboratory 1
 School of Tropical Medicine
 University of Puerto Rico
 San Juan, Puerto Rico

 Dr. O. G. Edholm 1
 Head, Division of Human Physiology
 Medical Research Council Laboratory
 Holly Hill, Hampstead
 London, NW 3
 England

FOREIGN ADDRESSES

Copies

British Naval & Army Medical 2
 Liaison Officer
 Bureau of Medicine & Surgery
 Building 4, Room 60A
 23rd & E Streets
 Washington, D. C.

DISTRIBUTION LIST FOR AMRL REPORTS

Category (B)

<u>ARMY</u>	<u>Copies</u>	<u>ARMY</u>	<u>Copies</u>
Commanding Officer Engineer Research & Development Laboratories Fort Belvoir, Virginia ATTN: Technical Documents Center	1	Commanding General Aberdeen Proving Ground, Maryland ATTN: Human Engineering Laboratory	1
President Board Nr 2, OCAFF Fort Knox, Kentucky	1	Commanding Officer Surgical Research Unit Brooke Army Medical Center Fort Sam Houston, Texas	1
Commanding Officer Frankford Arsenal Philadelphia, 37, Pennsylvania ATTN: Human Engineering Mission (LC)	1	President Board Nr 3, OCAFF Fort Benning, Georgia	1
<u>NAVY</u>		<u>NAVY</u>	
Commanding Officer U. S. Naval Medical Research Unit Nr 1 Bldg T-19, University of California Berkeley 4, California	1	Technical Information Division, Code 3-222A U. S. Naval Radiological Defense Laboratory San Francisco 24, California	1
<u>AIR FORCE</u>			
United States Air Force School of Aviation Medicine Radiation Laboratory University of Chicago 93 East 58th Street Chicago 37, Illinois	1		
<u>OTHER AGENCIES</u>		<u>OTHER AGENCIES</u>	
Chief, Radiation Branch National Cancer Institute Department of Health, Education & Welfare Bethesda 14, Maryland	1	Chief, Laboratory of Physical Biology National Institutes of Health Bethesda 14, Maryland	1
Librarian Arctic Health Research Center Post Office Box 960 Anchorage, Alaska	1	Research Library Brookhaven National Laboratory Associated Universities, Inc. Upton, L.I., New York	1
<u>FOREIGN ADDRESSEES</u>			
Dr. Alan C. Burton Department of Biophysics University of Western Ontario 346 South Street London, Ontario Canada	1		